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Comparative Phytotoxicity of the Fumonisins, AAL-Toxin and Yeast Sphingolipids in *Lemna minor* L. (Duckweed)

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Abstract. Fumonisin B_1 and AAL-toxin, both of which contain sphingolipid-like substituents, are water-soluble metabolites of *Fusarium moniliforme* and *Alternaria alternata*, respectively. These two toxins were compared to each other and to tetraacetylphytosphingosine (TAPS) and triacetyldihydrosphingosine (TADS) for effects on chlorophyll production and growth in *Lemna minor* L. (duckweed). Fumonisin B_1 (0.7 μg), TAPS, and TADS all produced parallel effects on growth rate and chlorophyll content; however, FB_1 did so at a 33-fold lower concentration. The AAL-toxin at 0.7 μg affects chlorophyll content more than plant growth (36% versus 73%, respectively), whereas at 3.3 μg concentration, the growth rate was less than 50% and chlorophyll content was reduced by 80%. By contrast, the hydrolysis product of FB_1 that does not contain the tricarballylic acid (TCA) substituent is 23 times less active, which suggests that this component somehow enhances activity. The yeast sphingolipids are completely acetylated and do not contain TCA groups but also affect chlorophyll content and growth rate of duckweed. However the effect was substantially less than with AAL-toxin and FB_1, which contain one and two TCA groups, respectively.

Recently, we reported on the phytotoxicity of six water-soluble metabolites of *Fusarium* or *Lemna minor* L. (duckweed) (Vesonder *et al.* 1992). Of the toxins tested, FB_1, a phytotoxic (Abbas *et al.* 1991, Mirocha *et al.* 1990) and mycotoxic (Bezuidenhout *et al.* 1988) metabolite of *Fusarium moniliforme*, was the most active. This toxin was isolated and characterized by Bezuidenhout *et al.* (1988) as 14,15-(1′,2′,3′-propane tricarboxylic acid)-2-amino-12,16-dimethyl-3,5,10-trihydroxyeicosane. AAL-toxins are structurally similar to fumonisins but are produced by a different fungus, *Alternaria alternata* f. sp. *lycopersici*. The AAL-toxins are host-specific to tomato (Gilchrist and Grogan 1976). The AAL-toxins were isolated and characterized by Bottini *et al.* (1981). Each AAL-toxin bears a simple TCA ester on either carbon 14 or 15 on the backbone chain of 1-amino-11,15-dimethyl-2,4,5,14,15-heptadecapentol. Additionally, the fumonisins and AAL-toxins contain sphingolipid-like groups (—CH(OH)—CH_2—CH(OH)—CH(NH_2)—CH_3). The sphingolipids which have the common structure (—CH(OH)—CH(NH_2)—CH_2OH) are found to accumulate in abnormal levels in the brain when certain pathological conditions persist (Hannun and Bell 1989; Morell and Braun 1972); *i.e.*, when the fumonisins induce neurotoxic disease in horses (Marasas *et al.* 1988). Neither their incorporation nor their role in the degeneration of the brain are understood.

In tomato, the target species of *A. alternata*, the AAL-toxin site of action is still an open question (Fuson and Pratt 1988). The fumonisins are inhibitors of sphingosine biosynthesis in cultured hepatocytes (Wang *et al.* 1991) and similarly, Kaneshiro *et al.* (1992) found the accumulation of sphingolipids in the yeast *Pichia ciferri*. However, the role of AAL-toxin and fumonisin in plants regarding sphingolipid biosynthesis and other biochemical events has yet to be determined.

This study compares the effects of the yeast sphingolipids, TAPS and TAD, FB_1, AAL-toxin and the aminopentol (AP_1) (hydrolysis product of FB_1) on growth and chlorophyll production of *Lemna minor* L. (duckweed). Structures of these metabolites are shown in Figure 1.

Material and Methods

Sphingolipids, Fumonisin, and AAL-Toxin

Sphingolipids TAD and TAPS were produced through yeast fermentation by the methods of Stodola *et al.* (1962). Fumonisin B_1 was produced by culture of *Fusarium moniliforme* on corn (Vesonder *et al.* 1990) and purified as previously reported by Vesonder *et al.* (1992). AAL-toxin was prepared as reported by Shier *et al.* (1991) from culture filtrate of *A. alternata* and subsequent purification by preparative TLC.

Fumonisin Hydrolysis Product AP_1

Fumonisin B_1 (25 mg) was saponified at pH 10 with sodium hydroxide for 4 h at room temperature (22–24). The reaction mixture was treated

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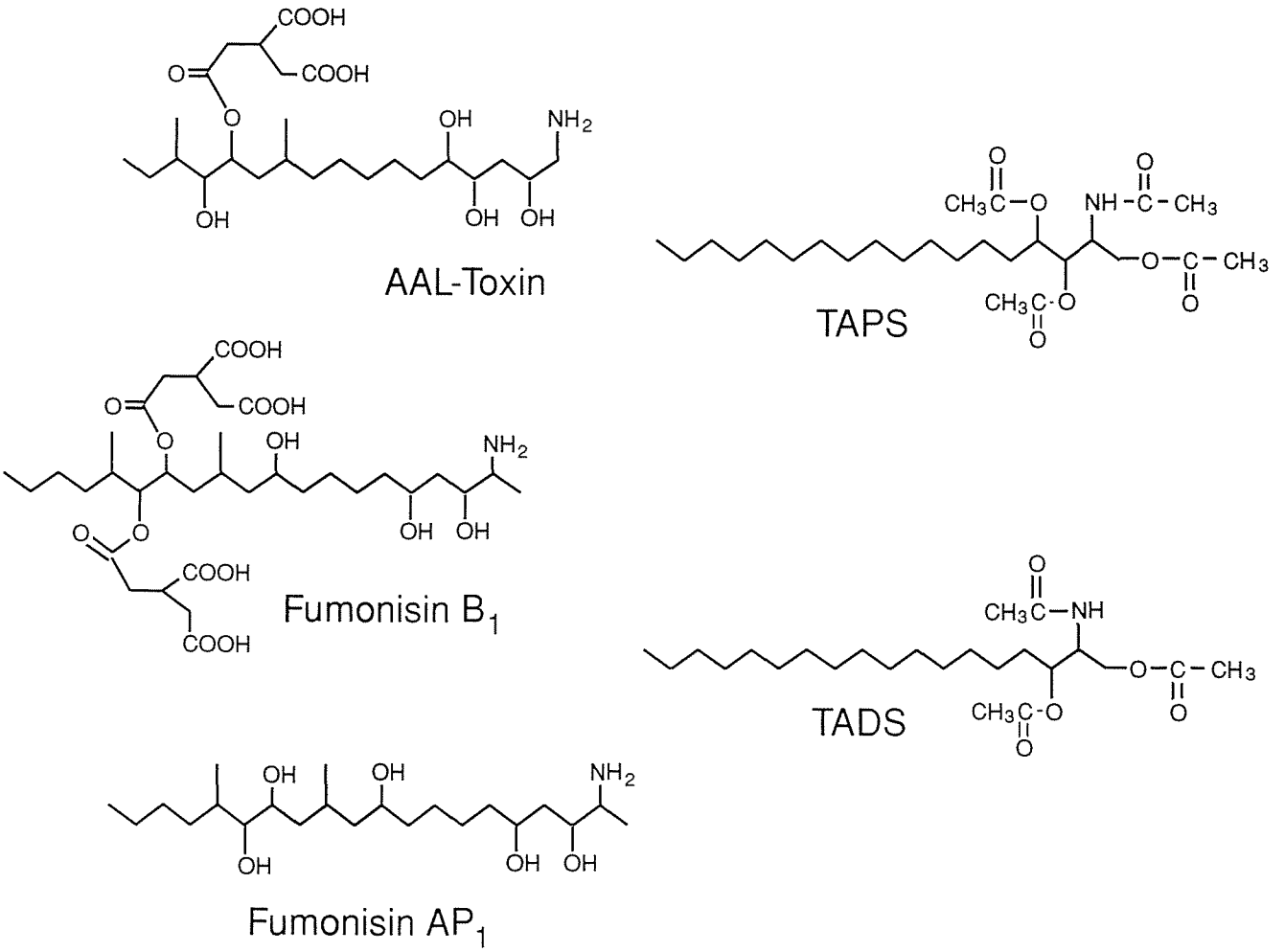

Fig. 1. Structures of AAL-toxin, fumonisin B_1, pentolamine AP_1, TAPS (tetraacetylphytosphingosine), and TADS (triacetyldihydrosphingosine)

in the following manner. First it was poured onto a XAD-2[2] (Sigma Chemical Co., St. Louis, MO. USA) column that was then washed with water until the effluent reached pH 6. Then the AP_1 was eluted with methanol. Methanol was removed from the eluate by evaporation and the remaining residue in CH_3OH:H_2O(10:90) was chromatographed on a C-18 reversed-phase low pressure column. The column was washed with a methanol and water gradient starting at 90% aq. methanol and ending with methanol. The appropriate fractions containing the AP_1, as assayed by TLC versus an authentic sample (W.C.A. Gelderblom, Research Institute for Nutritional Diseases, Tygerberg, South Africa), were combined. Approximately 7 mg of AP_1 was obtained.

Duckweed Bioassay

Duckweed bioassays were performed in three replicates for each toxin as previously described (Vesonder et al. 1992). Briefly, wells of sterile, plastic 24-well tissue culture plates containing 1.5 ml of sterile E medium and 3 *Lemna minor* L. fronds were treated with test metabolites. The plates were incubated for 10 days in a lighted growth chamber at 25°C, and then the fronds were counted. The total chlorophyll in the fronds was determined by extracting the fronds from each well with 1.5 mL 95% ethanol for six hours. This extract was removed, and a second 1.5 mL aliquot was added and incubated in the dark overnight. The ethanol extracts were combined and the chlorophyll content in each sample was determined spectrophotometrically at absorbances of 649 and 655 nm. The means and standard deviation were calculated for each set of wells. Growth rate was calculated as [log(final fronds) − log(initial fronds)]/number of days according to Einhellig *et al.* (1985).

Results and Discussion

The results presented in Table 1 demonstrate phytotoxic activity of the structurally related FB_1 and AAL-toxin and, possibly, also of yeast sphingolipids that do not contain the tricarboxylic acid moiety. The *Lemna minor* used in this study represents a microaquatic species that appears to be sensitive to these metabolites, and consequently useful for measuring phytotoxic effects on plant growth and chlorophyll production.

That chlorophyll contents of duckweed treated with either FB_1 or AAL-toxin were comparable for toxin concentrations of 0.7 μg/mL, indicates that both are effective phytotoxins. However, AAL-toxin exhibited less inhibition of growth rate as

[2]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Table 1. Effect of fumonisin B_1, AAL-toxin, and yeast sphingolipids on *Lemna minor* (duckweed)

Metabolite	Concentration (μg/ml)	Growth rate[a]	% of Control	Total chlorophyll[b]	% of Control
Fumonisin B_1[c]	0.0	0.091		2.37	
	0.7	0.043	47	0.84	41
	2.0	0.032	35	0.38	22
	3.3	0.018	20	0.33	16
	6.7	0.018	20	0.21	10
	10.0	0.000	0	0.13	6
AAL-Toxin	0.0	0.066		4.58	
	0.7	0.048	73	1.64	36
	3.3	0.022	33	0.82	18
	6.7	0.016	24	0.69	15
	10.0	0.004	6	0.30	7
Fumonisin AP_1	0.0	0.041		2.11	
	0.7	0.056	137	2.24	109
	3.3	0.043	105	2.18	106
	6.7	0.026	64	1.38	67
	16.7	0.016	56	1.08	53
Tetraacetylphytosphingosine	0.0	0.069		1.72	
	0.7	0.059	85	1.69	98
	3.3	0.050	73	1.70	98
	6.7	0.054	78	1.35	79
	16.7	0.042	61	1.19	69
	33.3	0.037	54	0.71	42
	66.7	0.025	37	0.03	2
Triacetyldihydrosphingosine	0.0	0.046		1.68	
	0.7	0.049	107	2.05	122
	3.3	0.039	85	1.24	74
	6.7	0.048	107	1.92	114
	16.7	0.032	70	1.09	65
	33.3	0.027	59	0.82	49
	66.7	0.020	44	0.00	0

[a] Average standard deviation = ± 0.007
[b] Average standard deviation = 17%
[c] Data from Vesonder *et al.* 1992

indicated by survival of 73% of the plants treated with 0.7 μg/mL whereas less than 50% of the plants survived the same level of FB_1. Two differences in the structures of FB_1 and AAL-toxin are that FB_1 contains one more TCA group and the amine group is on carbon-two instead of carbon-one. Similar structure/activity relationships for these toxins were reported by Shier *et al.* (1991) for established hepatoma (H4TG) and MDCK dog kidney cell lines. In addition, these same investigators reported that these mammalian cell lines are twice as sensitive to FB_2, which contains one less hydroxyl group than FB_1 and is less soluble in water (personal observation). In the comparison of duckweed to leaf cultures of pathogen-sensitive tomatoes, FB_1 is about 16 times more active to the former (Mirocha *et al.* 1990). The mechanisms of action of these toxins on these systems is not presently understood, but studies of sensitive bioassays may provide useful information for determining structure relationships.

Yeast extracellular sphingolipids TAPS and TAD were found to inhibit growth rate and chlorophyll content at concentrations of 33.3 μg/mL. These levels represent an increase of approximately 30-fold as compared to FB_1 and AAL-toxin. The yeast sphingolipids, which do not contain hydroxyl groups derivitized with TCA, but have acetates on each hydroxyl group and the amine group had less influence on the duckweed.

The AP_1 backbone of fumonisin B_1 obtained by base hydrolysis, which contains only hydroxyl groups and an amine group, inhibited chlorophyll production 53% and growth rate 56% at 16.7 μg/mL. This result indicates that the backbone of fumonisin is about 23 times less effective than FB_1. The major difference between the hydrolysis product and both FB_1 and AAL-toxin is the absence of the TCA moiety. This group appears necessary for enhancement of toxicity to the duckweed. The FB_1 seems to be more toxic than AAL-toxin, which might be attributable to the different amino attachment site and FB_1's additional TCA moiety. More information concerning the functional groups of FB_1 and AAL toxin is needed to resolve the contributions of the TCA group, the amino group, and the position of the hydroxyl groups toward toxicity. Apparently the TCA group is important for its effect on duckweed. Because it is not known whether this group affects the pH of the medium or simply binds trace essential metal ion(s), how the structural functionalities of these metabolites alter growth rate and chlorophyll content of *L. minor L.* (duckweed) is not known.

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